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SENSITIZED AND NONSENSITIZED VACCINES IN CHOLERA IMMUNIZATION

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Some clinical observers claim a relative superiority for sensitized vaccine over nonsensitized on the ground that sensitized cholera vaccine causes only slight symptoms when inoculated subcutaneously, while inoculation with nonsensitized vaccine is sometimes followed by local pain, fever and other reactions. If inoculation of sensitized cholera vaccine causes the formation of antibodies that we can trace in vitro, or of some other protecting agencies in the body against cholera bacilli, i. e., is more efficient or just as efficient as that of nonsensitized vaccine, and if the undesirable symptoms following the inoculation of sensitized vaccine are much milder than those following the inoculation of the nonsensitized, the latter obviously should not be used for prophylactic inoculation on human beings.

As early as 1901, Pfeiffer[1] published results obtained by immunization of rabbits with a mixture of cholera bacilli and cholera immune serum, testing fewer animals than we have used and employing as antigen for immunization a material somewhat different from the so-called "sensitized cholera vaccine." S. Yabe[2] reported the results of his experiment on 17 students in the Higher Normal School of Tokio. He noted that the bacteriolytic activity tested in vitro of the serums of 9 students, in whom he had previously (16 days before) injected two times his nonsensitized cholera vaccine subcutaneously for prophylaxis, was much higher than that of the serums of 8 students injected with the same amount of his sensitized cholera vaccine under the same experimental conditions. Later, however, he makes no mention of the valuable results obtained in his experiments on human beings.[3]

In an attempt to determine, if possible, the relative value of sensitized and nonsensitized vaccines in cholera immunization, I made the following experiments.

PREPARATION OF VACCINE

Cholera bacilli were cultivated on ordinary slightly alkaline agar-agar mediums in large tubes, incubated at 36 C. for 18 hours. The bacilli were gathered by means of a large platinum loop into a weighing bottle (avoiding mixing the condensed water as much as possible) and were weighed on a chemical balance.

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[1] Deutsch. med. Wchnschr., 1901, pp. 867 and 891.

[2] Tokio Iji Shinshi, 1916, No. 1997, p. 1.

[3] Shiga, K., Takano, R., and Yabe, S.: Kitasato Arch. of Exper. Med., 1918, 2, p. 1.

Sensitized Vaccine.—To each 10,000 gm. of cholera bacilli, made into homogeneous emulsion with sterile salt solution of 100 c c or 150 c c, are added 10 c c of sterile inactive anticholera horse serum, the agglutination titer of which is over 20,000. This was stirred with a sterile glass rod to insure thorough mixture. Marked agglutination occurred almost instantaneously. After two hours at 37 C., when agglutinated bacilli had settled almost entirely, the contents were again stirred up and the whole suspension was kept for another two hours at 37 C. It was then subjected to strong centrifugalization, and the mass of bacilli was separated and suspended in 1,000 c c of sterile normal salt solution, to which 0.5% carbolic acid was added. The carbolized emulsion of sensitized germs was placed over night at 37 C. and cultivation tests made to make sure that the vaccine was sterile. For ordinary prophylaxis we used both sensitized and nonsensitized cholera vaccines of the concentration of 2 mg. of bacilli in 1 c c.

Nonsensitized Cholera Vaccine.—This was prepared according to Kolle.[4] Throughout the work we used both kinds of vaccines of the same date for each series of comparative experiments.

To determine the toxicity of each vaccine, guinea-pigs weighing between 150-230 gm. were used. Both kinds of vaccine were injected intraperitoneally or subcutaneously, and the smallest amount causing death within, or nearly within, 24 hours observed. Of the sensitized cholera vaccine, 125-150 mg. injected intraperitoneally, caused death in 12-15 hours, while 15-20 and 18-20 mg. of nonsensitized cholera vaccine caused the death of the test animals in 12-16 and in 20 hours, respectively. Of sensitized vaccine 200-250 mg., injected subcutaneously, caused death of the test animals in 28 hours; 75-100 mg. of nonsensitized cholera vaccine, injected subcutaneously, caused death in 29 hours.

The lethal dose of living cholera bacilli sensitized for two hours at 37 C. and then centrifugalized was found to be 5-10 mg., injected intraperitoneally; this dose proved fatal in 35 hours. Intraperitoneal injection of 0.50-0.75 mg. of living cholera bacilli caused death in 26 hours. Of living cholera bacilli 5-7 mg. injected subcutaneously, caused the death of test animals in 27 hours.

The lethal doses of cholera vaccines varied in accordance with the so-called virulence of the respective cholera strain. For example, the lethal dose of nonsensitized cholera vaccine from strain "Yanagiwara" 1 was found to be 18-20 mg. (the lethal dose of the cholera bacilli being 0.75-1 mg.). The lethal dose of strain "Yanagiwara" 3 was 10-12 mg., while the lethal dose of the cholera bacilli was 0.25 mg., illustrating the observation previously reported that cholera vaccine made from more virulent strains is more toxic than that from less virulent strains.

Some investigators may attribute the marked reduction of toxicity of cholera vaccine by means of sensitization to the "washing" in the course of sensitization. Certainly some of the loss in, or diminution of, bacillary substance may be due to the sensitization and washing (centrifugalization), but it is obviously a mistake to assume that during the process of sensitization of living cholera bacilli, the bacillary substances are diminished to 1/8 or 1/7, reducing the toxicity of sensitized cholera vaccine to 1/8 to 1/7 of that of nonsensitized vaccine.

We noticed that the loss of bacillary substances, owing to sensitization and washing by means of centrifugalization, tested by the nitrogen determination method of Kjeldahl, was not so large as the reduction of toxicity of vaccine examined by animal tests. Some cholera bacilli may escape, or die, or lose their toxic substances during sensitization and washing. This loss, however, would account for only a small part of the tremendous difference between the lethal doses of living bacilli sensitized and nonsensitized.

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[4] Deutsch. med. Wchnschr., 1897, 1, p. 4.

Are toxic properties of the bacillary substances neutralized in the process of sensitization, or does the specific anticholera serum used in the sensitization exert some influence on the bacilli and favorably prepare the way for phagocytosis in vivo?

To determine the protective efficiency of inoculation of cholera vaccine, sensitized and nonsensitized, against cholera infection, guinea-pigs, 150-200 gm., and rabbits were inoculated, both intraperitoneally and subcutaneously, with vaccine. To test the degree of active immunity thus produced, we used principally (1) the infection test and (2) the serum test in vitro of the amount of bacteriolytic substance contained in the serum of the inoculated animals.

In 12 series, using from 3 to 6 animals in each series, 1 c c of 1:100 dilution of cholera vaccine was inoculated intraperitoneally, employing in each series a test dose of twice the lethal dose of cholera bacilli, sensitized and nonsensitized. The infection test was made from 3 to 24 hours after the inoculation, and all the animals survived with the exception of those in series 9; 3 guinea-pigs died when the infection test was made 3 hours after the injection of nonsensitized cholera vaccine. In series 13, in which 5 guinea-pigs were inoculated with nonsensitized cholera vaccine and the test made 3 hours after inoculation, 1 died and 4 survived. In series 14, using sensitized vaccine with the same number of guinea-pigs, 2 survived and 3 died in the same period after the inoculation.

To test the bacteriolytic activity and the agglutination of serum from guinea-pigs previously inoculated with cholera vaccine, sensitized and nonsensitized, two guinea-pigs were inoculated intraperitoneally with 1 c c of 1:100 dilution of sensitized vaccine and tested after 3, 5, 7, 24, 48 and 72 hours. Bacteriolysis in vitro was negative with 0.1 c c of the serum. Using nonsensitized vaccine and testing the guinea-pigs at the same periods, bacteriolysis in vitro was negative with the same amount of serum. Agglutination in all these tests was negative in 1:4 dilution.

No marked difference in the efficiency of inoculation of cholera vaccine of either kind was apparent. It is possible that there may have been some individual variations in resistance against intraperitoneal cholera infection among the guinea-pigs.

Guinea-pigs previously inoculated intraperitoneally with 1 c c of 1:10 dilution of the original vaccines, did not survive infection with three or more than three times the lethal dose of living cholera bacilli administered intraperitoneally 48 hours, 4 days and even 6 days after the vaccine inoculation. In this case we could not see, of course, any difference in the efficiency of either cholera vaccine.

The determination of a suitable or adequate test dose constituted ultimately the hardest part of our experiments. The larger test doses that we used in the standardization of antibacterial serum by means of the infection test, seemed to be too large for our present purpose, because the amount of antibodies in the serum of guinea-pigs, or rather the degree of protecting power in general, after the inoculation of cholera vaccine, is not so abundant as in the case of testing of immune serum. The futility of conducting an infection test of this kind with inadequate or improperly chosen test doses of living cholera bacilli was emphasized in my discussion of the work of S. Yabe.[5]

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[5] Tokio Iji Shinshi, 1916, No. 2.

The protective power in animals inoculated against intraperitoneal infection with cholera bacilli of double the lethal dose seems to consist of some agency combined with the bacteriolytic and agglutinating substance in the serum of inoculated animals.

The efficiency of the subcutaneous inoculation of cholera vaccine, sensitized and nonsensitized, on guinea-pigs was observed by making infection tests and determining the bacteriolytic activity of the serum of animals previously inoculated with 1 c c of 1 : 100 dilution of the original cholera vaccine sensitized and nonsensitized. On a series of 28 animals we noted (1) that the bacteriolytic activity tested in vitro was negative with 0.1 c c of serum of both series of guinea-pigs inoculated with both kinds of vaccine, respectively, even on the tenth day after inoculation; (2) that the agglutination titer of serum of both series is lower than 1 : 4 dilution; (3) that even on the eighth day after inoculation the animals of both series could not show enough protecting power against the intraperitoneal infection with double the lethal dose of cholera bacilli; (4) that no difference in protecting efficiency of inoculation of sensitized or nonsensitized cholera vaccine against intraperitoneal infection was detected.

Using subcutaneous inoculation of a larger amount of cholera vaccine of both kinds than in the previous experiment, we obtained somewhat better results. A rather marked superiority in protecting power of animals inoculated with nonsensitized cholera vaccine was evident in comparison with that of animals inoculated with the same dose of sensitized cholera vaccine. The higher protecting power of guinea-pigs against the intraperitoneal infection with 1.5-2.5 lethal doses of cholera bacilli was especially marked and could be seen 5 days after subcutaneous inoculation.

In the early stage of immunization by means of subcutaneous inoculation of 1 c c (2 mg.) of cholera vaccine, sensitized as well as nonsensitized, the protecting power is so small that the animals are unable to survive even one lethal dose of cholera bacilli, while in intraperitoneal injections of 1 c c of 1 : 100 dilution of original vaccine of both kinds, as early as the fifth hour after the inoculation, all animals survive the intraperitoneal injection with double the lethal dose of living cholera bacilli. A test of more than one lethal dose of cholera bacilli is therefore entirely useless, at least in the early stage of immunization of guinea-pigs by subcutaneous inoculation, for the purpose of determining the difference in efficiency of the two types of cholera vaccines.

With a test dose of more than three lethal doses of cholera bacilli, all infection tests were negative even after 9 days of subcutaneous inoculation.[6] In attempting to determine the difference in efficiency of subcutaneous inoculations of cholera vaccines of both kinds by means of infection tests, the test doses, therefore, must not exceed 2.5 lethal dose of cholera bacilli.[5]

Rabbits were inoculated subcutaneously with cholera vaccines to determine the bacteriolytic activity of the serum. Of a 1 : 30 dilution of the original vaccines, sensitized and nonsensitized, respectively, 1 c c was given subcutaneously to each of the 5 rabbits for the first injection. This was followed after 3 days' interval by a second injection of 1 c c of a 1:15 dilution of the original vaccines. After 7 days a blood sample was taken from the rabbits (1 rabbit died from an unknown cause on the fifth day after the second injection) and the bacteriolytic activity of the separated serum was tested in vitro by the plate method according to Neisser and Wechsberg.

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[6] In this connection it may be of some interest to refer to the work of Otto Schöbl and C. S. Panganiban (Philippine Jour. Sc., Manila, 1917, Sec. B, 12, p. 43). In their study on experimental cholera carriers and immunity, there was no apparent shortening of the duration of cholera carriers in guinea-pigs effected by preventive vaccination and vaccine therapy. Guinea-pigs immunized by the subcutaneous administration of graduated doses of suspension of killed cholera vibrios and infected at intervals varying from three to twelve days after the infecting dose, gave positive cultures of cholera vibrios from the gallbladder, duodenum, ileum and cecum in almost every case.

The bacteriolytic activity of the serum from rabbits inoculated subcutaneously with sensitized cholera vaccine is by far weaker than the bacteriolytic activity of serum from rabbits inoculated with nonsensitized cholera vaccine. This result agrees with the result obtained by S. Yabe in his study on 17 students.[2] According to his report the highest bacteriolytic activity of serum from 8 students inoculated with his sensitized cholera vaccine (positive bacteriolysis with 0.00005 c c of serum) coincided with the lowest titer of the serum obtained from 9 students inoculated with his nonsensitized cholera vaccine under the same experimental conditions. He inoculated the students twice subcutaneously for prophylaxis, and on the sixteenth day after the second injection blood samples were taken to determine the bacteriolytic power.

Testing 10 rabbits, 5 inoculated subcutaneously with nonsensitized vaccine and 5 with sensitized vaccine, the agglutinative power of the serum from rabbits inoculated with the nonsensitized vaccine was found generally to be remarkably higher than that of serum from rabbits injected with sensitized vaccine. This is analogous to the result obtained by K. Imai[7] in his investigation on the sensitized typhoid, paratyphoid A and paratyphoid B vaccines.

Though the bacteriolysin alone may not be the principal agency which confers the protecting power on animals inoculated with cholera vaccine, we seem warranted in concluding that the higher bacteriolytic activity may go hand in hand with a greater protecting power for animals. It is therefore probable that the nonsensitized vaccine that causes greater production and appearance of bacteriolytic substance in the blood, when injected subcutaneously in rabbits as well as in human beings (according to S. Yabe), is superior in efficiency to the sensitized vaccine which does not produce as much bacteriolysin in the blood.

CONCLUSIONS

During the process of sensitization, the toxicity of cholera vaccine as tested on guinea-pigs is diminished greatly. This reduction of toxicity is caused not only by the washing, but also by the specific influence of anticholera serum on the toxic substances of cholera bacilli.

Further investigation and observation will determine whether there is a reduction of toxicity of cholera vaccine by sensitization for human beings.

Careful selection of the test dose is a fundamental necessity for the infection tests on guinea-pigs that have been inoculated with cholera vaccines, either intraperitoneally or subcutaneously.

In order to determine the comparative value of sensitized and nonsensitized cholera vaccine on intraperitoneal inoculation, further tests, using twice the lethal dose of cholera bacilli within 3-5 hours after the intraperitoneal inoculation, must be employed on guinea-pigs.

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[7] Kaigun, Gunidan, 1916, No. 12.

Nonsensitized cholera vaccine is markedly superior to sensitized cholera vaccine in protecting power for guinea-pigs inoculated subcutaneously. Nonsensitized cholera vaccine produces more bacteriolytic substance in the blood of rabbits inoculated subcutaneously than sensitized cholera vaccine. S. Yabe observed this same phenomenon in his experiments with cholera vaccines on human beings.

Nonsensitized cholera vaccine, inoculated subcutaneously, is much more effective than the sensitized.